

Running title: Corn Bran Arabinoxylans and Immunomodulatory Activity

Characterization of Nitric Oxide Modulatory Activities of Alkaline-Extracted and Enzymatic-Modified Arabinoxylans from Corn Bran in Cultured Human Monocytes

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1 **ABSTRACT**

2 The ingestion of foods and food-derived substances that may mediate the immune
3 system is widely studied. Evidence suggests cereal arabinoxylans (AXs) have
4 immunomodulatory activities that may impart health benefits in terms of immune
5 enhancement. This study extracted AXs from corn bran using alkali and developed a
6 modification process using three endoxylanases to obtain fractions of lower molecular
7 weight ranges. In vitro studies showed extracted and modified AXs significantly ($P <$
8 0.05) elevated nitric oxide (NO) synthesis by the human U937 monocytic cell line
9 (ranging from 53.7 ± 1.1 to 62.9 ± 1.2 μM per million viable cells) at all concentrations
10 tested (5-1000 $\mu\text{g/ml}$), indicative of immune enhancement compared to an untreated
11 control (43.7 ± 1.9 μM per million viable cells). The study suggested the dose range and
12 Mw distribution of AXs are key determinants of immune-modulatory activity. AXs in
13 the low Mw range (0.1KDa-10KDa) were the most effective at inducing NO secretion
14 by U937 macrophages at low AX concentration ranges (5-50 $\mu\text{g/ml}$) with NO
15 production peaking at 62.9 ± 1.2 μM per million viable cells with 5 $\mu\text{g/ml}$ of AX ($P =$
16 0.0009). In contrast, AXs in the high Mw range (100-794KDa) were most effective at
17 inducing NO at high AX concentration ranges (500-1000 $\mu\text{g/ml}$) with NO production
18 reaching a maximum of 62.7 ± 1.3 μM per million viable cells at 1000 $\mu\text{g/ml}$ of AX (P
19 = 0.0011). The findings suggest that dietary AXs from corn bran may heighten innate
20 immune responses in the absence of infection or disease.

21
22 **Keywords:** Arabinoxylan; Corn bran; Molecular weight (Mw); Immune-modulatory
23 activity; in vitro

25 INTRODUCTION

26 The immune system plays a pivotal role in the protection and maintenance of human health ¹. Several
27 factors including malnutrition and unhealthy lifestyles (e.g. poor diet) can disturb human immune functions
28 ²⁻⁴. Consequently, the ingestion of foods and food-derived substances that may enhance the immune system
29 is widely studied. Dietary intervention is considered an efficient way of preventing a decline in immune
30 functions and reducing the risk of infection or cancer ^{5,6}. Some dietary fiber-derived food substances (such as
31 β -1,3-glucan, β -1,6-glucan and α -1,6-mannan) have been discovered that show immune stimulation activity
32 ^{7,8}. Arabinoxylan (AX), an important hemicellulose found in the outer-layer and endosperm cell walls of
33 cereals, is a dietary fiber. In vivo studies have reported that cereal AXs can modulate both the innate (typical
34 of natural killer cells and monocytes/macrophages) and adaptive (typical of T and B cells) immune activities
35 after oral administration in mice and chicken ⁹. For example, dietary supplementation of AXs (at 100-200
36 mg/kg body weight each day for 15 days) has been shown to induce murine macrophage activation and
37 phagocytosis ^{10, 11}.

38 Although the exact mechanism of how dietary fibers, including AXs, modulate the immune response is
39 not well understood, several studies have shown that dietary fibers can be taken up by microfold cells,
40 macrophages and dendritic cells in the intestines and transported to lymph nodes, thus providing a mechanism
41 for the distribution of AXs in the body. Moreover, direct interaction of fibers with colonic epithelial cells or
42 innate immune cells (such as macrophages) can mediate cytokine production leading to inflammatory and

43 anti-tumor effects ^{8, 12, 13}. Macrophages are present in high numbers in the intestine and are frequently in close
44 proximity with epithelium cells ¹⁴. A previous study demonstrated that rice-derived and wheat-derived AXs
45 are dietary fibers that are taken up by gastrointestinal macrophages and transported to the spleen, lymph
46 nodes and bone marrow ¹². Furthermore, these studies also showed that dietary AXs can interact with murine
47 intestinal macrophages and stimulate immune responses in vivo ^{12, 14}. Once transported to tissues such as the
48 spleen, lymph tissues and bone marrow dietary-derived AXs can modulate inflammatory responses in non-
49 intestinal, peripheral tissues by interacting with resident inflammatory cells (including tissue macrophages)
50 or with bone marrow-derived leukocytes (e.g. monocytes) that may be subsequently recruited from
51 circulation to sites of infection, injury or disease. Indeed, a human study involving 80 human participants
52 showed that oral consumption of AX (3 g daily for 8 weeks) as a dietary supplement significantly increased
53 interferon gamma (IFN- γ) production in circulating leukocytes of healthy adults ¹⁵, thus confirming non-
54 intestinal inflammatory effects of AXs on peripheral blood mononuclear cells in vivo.

55 The structural properties of AXs are affected by the plant source, extraction processes, modification and
56 purification methods. This results in AX extracts with varying molecular size and degree of branching ¹⁶.
57 Intestinal innate immune cells, such as macrophages, can interact with an array of AXs with differing
58 structural properties typically found in the diet ¹². Thus, it is essential to investigate the relationship between
59 structural details and biological activity. Recent in vivo and in vitro studies suggest the immunological
60 properties of wheat AXs might be influenced by structural features including molecular characterization,

61 degree of branching and monosaccharide compositions ¹⁰. However, there has been no clear consensus as to
62 which structural properties of AXs modulate immune function. Thus, the precise structure-activity
63 relationship of AXs remains to be elucidated. Corn bran is a by-product of corn starch processing, which has
64 been identified to be a cost effective source of AXs (25-30% by content) for which extraction methods have
65 been developed ¹⁷⁻¹⁹. However, very little data exists on immunomodulatory properties of corn-derived AXs.
66 Endoxylanases and cellulase are commonly used to extract and degrade AXs from cereal cell wall tissue ¹⁶.
67 Endo- β -1,4-xylanases (EC 3.2.1.8) cleave the β -glycosidic bond between two β -(1,4) D-xylopyranosyl units
68 ^{20, 21}. Endo- β -xylanases are mainly classified into two glycoside hydrolase (GH) families, namely GH 10 and
69 GH 11. Endoxylanases from the GH11 family are commonly used to hydrolyze AXs because of their higher
70 substrate specificity compared to GH 10 endoxylanases ^{21, 22}. Three GH 11 endoxylanases from *Thermomyces*
71 *lanuginosus*, *Neocallimastix patriciarum* and *Penicillium funiculosum* have been characterized and used in
72 different studies for isolation and modification of cereal hemicellulose ²³. However, the effects of these three
73 enzyme modification processes on the molecular structures of corn bran AXs have not been well documented.
74 Thus, this investigation used chemical extraction and enzyme modification processes for modifying the
75 chemical structures of AXs extracted from corn bran. Subsequently, corn-derived AXs were assessed for their
76 immune-modulating effects on human macrophages in a monocytic (U937) cell line by measuring nitric
77 oxide (NO) levels. NO mediates inflammation and host defenses by regulating signaling pathways and
78 transcription factors, vascular responses, leukocyte adhesion and transmigration, cytokine expression as well

79 as proliferation and apoptosis²⁴. The U937 cell line is frequently used to model human macrophages instead
80 of using non-human (animal) macrophage cell lines such as RAW264.7 and has been previously used to
81 investigate the effects of cereal AXs (at concentrations ranging from 1 to 500 µg/ml) on several immune
82 activities in vitro, including NO production²⁵.

83

84 MATERIALS AND METHODS

85 **Chemicals.** The sample of dried corn bran (moisture content 4.5%) was kindly provided from the
86 Chinese Academy of Agricultural Sciences (Beijing, China). There were three endo-1,4-β-xylanase products
87 (EC 3.1.2.8) used in the modification of AXs, which were Pentopan Mono BG (P-BG, 2500 U/g) from
88 *Thermomyces lanuginosus* (Novozyme, Bagsvaerd, Denmark), E-XYLNP (1000-1500 U/mg) from
89 *Neocallimastix patriciarum* (Megazyme, Bray, Wicklow Ireland) and Optimash VR (O-VR, 3150 U/g) from
90 *Penicillium funiculosum* (Genencor, Leiden, Netherland). The 1,4-α-D-glucan glucanohydrolase (α-Amylase
91 heat stable, ≥500 U/ml) from *Bacillus licheniformis* and proteinase (≥500 U/ml) from *Aspergillus melleus*
92 were purchased from Sigma-Aldrich, Gillingham, United Kingdom. D-(+)-xylose (≥ 99%) for AX extraction
93 yield determination was purchased from Acros Organics (Loughborough, UK). Eight pullulan (without side
94 chains) standards of varying molecular weights (5–800KDa) were purchased from Shodex (Shanghai, China).
95 D-glucose (≥ 99.5%), D-xylose (≥ 99%), L-arabinose (≥ 99%), and D-galactose (≥ 99%) were purchased from
96 Sigma–Aldrich (Gillingham, UK). U937 cells were purchased from the Public Health England Culture

97 Collections ²⁶. Lipopolysaccharide (LPS) (*E. coli* serotype O111:B4, 25mg) was purchased from Sigma
98 Aldrich, UK. RPMI-1640 cell culture medium with L-Glutamine was purchased from Lonza (Verviers,
99 Belgium). Foetal bovine serum (impurities \leq 10 EU/mL endotoxin) and penicillin-streptomycin (10,000
100 units penicillin and 10mg streptomycin/ml) were purchased from Sigma-Aldrich (Gillingham, USA).

101 **Extraction and purification process of AXs from corn bran.** The alkaline extraction of AXs from
102 corn bran was based on Doner, et al. ¹⁹. Milled corn bran (300g) was mixed in 2000g of distilled water (1.5:10,
103 w/w) using a hand blender (800W, WSB800U) for 45s. The mixture was adjusted to pH 7.0 before adding
104 780 μ l Termamyl α -amylase and placing in a 90°C shaking (100 rpm) water bath for 1h. The mixture was
105 then boiled for 15 min to inactivate the enzyme. Following subsequent centrifugation of the mixture (6000 x
106 g, 20min), the residue was recovered and oven-dried overnight at 45°C to obtain de-starched corn bran. De-
107 starched corn bran samples were mixed in distilled water (1:10, w/w). Appropriate amounts of NaOH were
108 added to make up 1%, 2%, 4% and 8% w/w NaOH concentrations. The mixtures were boiled for 1h with
109 stirring, followed by centrifugation (6000 x g, 20min). The supernatants were recovered and weighed for
110 further precipitation. The ethanol precipitation method was used from Li, et al. ²⁷. Briefly, 600ml AXs-
111 containing supernatant was mixed with 400 μ l proteinase at 60°C for 1h to remove protein. The solution was
112 then placed in boiling water bath for 15min to inactivate the proteinase followed by centrifugation (6000 x g,
113 20min). The supernatant was collected and mixed with 1.4L of 70% (v/v) ethanol. The mixture was kept at
114 4°C overnight followed by centrifugation (6000 x g, 20min). After centrifugation, the precipitate was washed

twice with 20ml aliquots of ethanol and re-suspended in 20ml acetone and washed for 1min. After washing, the precipitate was dried in an oven overnight at 45°C to obtain the alkaline extracted AX samples (AEAXs). The AEAXs were milled using an analytical mill (IKA A11 Basic, Guangzhou, China, 50/60Hz, 160W).

Modification process. A 1/25 (w/w) solution of AEAX was prepared by adding 3g AEAX (8% NaOH extracted) to 72g distilled water. Three different endo-1,4-β-xylanase products (E-XYLNP, P-BG, O-VR) were used to modify the AEAX sample (subsequently referred to as E-AEAX) using two different incubation times of 24h and 48h. Briefly, to each AEAX solution, 0.03g of enzyme (corresponding to 400 ppm) was added and mixed well. The optimum pH and temperature of each enzyme was provided according to studies of Malunga and Beta²⁸ and Li, et al.²⁷, together with the manufacturer instructions. The conditions of each enzyme treatment were set as pH 6.0, 50°C and 24h/48h for E-XYLNP treatment; pH 4.5, 50°C and 24h/48h for P-BG treatment; and pH 4.0, 50°C and 24h/48h for O-VR treatment. After 24h/48h of enzymatic treatment, the mixture was placed in a boiling water bath for 15min to inactivate the enzyme. The water content of the E-AEAX sample was then removed by rotary vacuum evaporation followed by drying overnight in a 45°C oven to form a dried residue that was milled with an analytical mill. Chemical reagents containing nitrate or nitrite were avoided in the alkaline extraction or the enzyme modification process in order to prevent contamination in the subsequent in vitro NO stimulation assay.

Analysis of AX extraction yields. A standard curve of xylose was constructed using the method described by Douglas²⁹ for determination of the xylose content of corn bran and extraction supernatants. In

turn, these measurements were used to calculate the content of AX in supernatants based on their A/X ratio as tested in monosaccharide composition analysis. A coefficient of 0.88 was used to correct for hydration water³⁰ in the equation $AX\% = Xylose\% \times (1+A/X) \times 0.88$. The AX content in the extraction supernatant was then used to calculate the extraction yield of AX from raw material based on the weight of the supernatant and raw material according to the equation: $AX \text{ extraction yield } (\%) = AX\% \times \text{weight of supernatant(g)} \div \text{weight of raw material(g)} \times 100$.

Analysis of AX sugar compositions. The monosaccharide compositions of AX samples were analyzed by following a method developed from Li, et al.³¹ whereby 1ml of H₂SO₄ (1M) was added to 20mg dried AX sample. The mixture was hydrolyzed for 2h at 100°C in a glycerin bath followed by a 20-fold dilution (1mg/ml) in HPLC grade water. The pH of the diluted solution was adjusted to 6.5-7.2 with 1M NaOH. The solution was then filtered through a 0.45µm nylon membrane for high-pressure liquid chromatography (HPLC) analysis. Isocratic elution using HPLC water as the mobile phase was conducted on a JASCO RI-2031 Refractive Index (RI) detector and Phenomenex ThermaSphere TS-130 column. Temperature of treatment was 85°C in all cases.

Analysis of AX molecular weight distributions. Size exclusion HPLC (SE-HPLC) test was used to determine the molecular weights and size distribution of dried AXs samples, according to the methods described by Li, et al.³¹ and Stoklosa and Hodge³². The average degree of polymerization (avDP) was calculated by dividing the apparent peak molecular mass by the molecular mass of anhydropentose sugars

151 (Mw=132Da)³³. Mobile phase was prepared by dissolving 17g NaNO₃ and 0.65g NaN₃ in 2L of HPLC grade
152 water. Eight pullulan standards with molecular weights in the range 5,000-800,000Da were used to construct
153 a standard curve. The standard and dried AX samples were dissolved in the mobile phase to form 0.5
154 and 2 mg/ml solutions, respectively, and gently stirred overnight prior to filtering through a 0.45µm nylon
155 membrane for SE-HPLC analysis. Isocratic elution was conducted on a JASCO RI-2031 refractive index (RI)
156 detector (Jasco Corporation, Tokyo, Japan) together with BioSep-SEC-S 4000 and BioSep-SEC-S 3000
157 columns (Phenomenex, Macclesfield, UK). Two continuous columns were connected in series (starting with
158 BioSep-SEC-S 4000) to improve the peak shape of the AXs samples. Running time was 60min per sample
159 and flow rate was 0.6 ml/min throughout.

160 **Human U937 Cell Culture.** Complete cell culture medium was prepared from RPMI-1640 with L-
161 glutamine (Lonza, Belgium), 10% foetal bovine serum (FBS) and 2% penicillin-streptomycin (P/S). The
162 human U937 macrophage cell line was grown in complete culture medium using sterile tissue culture flasks
163 under sterile conditions in an incubator at 37°C with 5% CO₂. The cells were sub-cultured every 2 days and
164 only used for experiments if viability was $\geq 90\%$.

165 **Polysaccharide medium preparation.** The 10mg AEAX sample from the alkaline extraction (NaOH
166 8%) of corn bran, the 10mg E-AEAX sample from the enzymatic modification (O-VR 48h) and 1ml LPS
167 (5mg/ml) were added to culture medium (RPMI-1640 with 5% FBS) to form a total volume of 10ml and
168 solubilized for 24h at 37°C prior to filtration through a 0.45µm sterile filter. Solubilized samples were diluted

169 further in culture medium for subsequent cell culture treatments according to concentrations (5 µg/ml, 50
170 µg/ml, 500 µg/ml, 1000 µg/ml) used in similar published studies ^{25, 27}.

171 **Cell viability and growth analysis.** Cell growth and viability of U937 cells following treatment with
172 AX samples and LPS were assessed by cell count and trypan blue uptake method ³⁴. U937 cells were
173 centrifuged (1000g for 10min) and re-suspended in culture medium (RPMI-1640 with 10% FBS) such that
174 the density of live (viable) cells was set at 1×10^6 /ml. A 100 µl cell suspension was pipetted in each well of a
175 96-well microplate. Sterile AX and LPS samples prepared in culture medium at three high concentrations
176 (50, 500 and 1000 µg/ml) were pre-warmed to 37°C before adding 100µl to six appropriate wells containing
177 U937 cells and mixed thoroughly. After 24h incubation (37°C, 5% CO₂), 40µl of cell fluid was added to an
178 equal volume of trypan blue (Sigma-Aldrich, UK). The number of trypan blue stained (non-viable) and non-
179 stained (viable) cells in each sample were counted using a TC10 automated cell counter (Bio-Rad, UK). The
180 percentage (%) viability of cells was calculated as the number of viable cells divided by total (viable and
181 non-viable) cells multiplied by 100.

182 **Nitric oxide (NO) stimulation assay.** This assay was used to evaluate the ability of AX samples to
183 induce NO production in the cell line U937 using Griess reagent. Griess reagent was freshly prepared
184 immediately before use in the NO stimulation assay according to the method of Dawson and Dawson ³⁵ and
185 Griess ³⁶. Griess reagent is made up by two components. Component A is 37.5mmol/L sulphanilamide with
186 deionized water in 6.5mol/L HCl by 1:1 (v/v). Component B is N-1-napthylethylenediamine dihydrochloride

(NEED) in deionized water at 12.5mmol/L. Both NEED and sulphanilamide were purchased from Sigma Aldrich, UK. Various immune cell lines including macrophages have been shown to produce elevated levels of NO in response to immune-stimulants such as LPS and cytokines³⁷. Thus, LPS was used as an appropriate positive (polysaccharide) control in this study. A 50µl suspension of U937 cells was added to an equal volume (50µl) of sterile AX or LPS diluted in culture medium to give a series of increasing concentrations (5 µg/ml, 50 µg/ml, 500 µg/ml, 1000 µg/ml) in wells of a 96-well microplate prior to 24h incubation (37°C, 5% CO₂) as described previously^{25,27}. Untreated controls were included on the microplate by adding 50µl culture medium (in place of AX/LPS) to appropriate wells containing 50µl of viable U937 cells. Background levels of nitrite and/or interference from nitrate present in AX samples were internally controlled for within the assay by taking in account the direct activity of AX samples in wells containing 50µl culture medium and an equal volume of diluted AX sample in the absence of U937 cells. All experimental samples were evaluated in triplicate with appropriate adjustments for background levels as indicated above. After 24h incubation, 50µl of Component A was added to each well and mixed. The microplate was incubated at room temperature for 10 minutes before adding 50µl of Component B to each well and thoroughly mixing. The microplate was then incubated at 4°C for 20 minutes. The absorbance of each well was then measured at 540nm using a microplate reader (Synergy HTX Multi-Mode Reader, Biotek, UK). A nitrite standard reference curve was used to quantitatively determine the indicative concentration of NO in the experimental wells. Nitrite standards were prepared using sodium nitrite (Sigma Aldrich, UK) diluted in medium (RPMI-1640 with 10%

205 FBS) at a range of concentrations (0, 0.1, 1.0, 10, 25, 50, 80 and 100 μ M).

206 **Statistics.** Experiments, unless otherwise stated, were performed in triplicate and analyzed by one-way
207 ANOVA followed by a post-hoc Tukey test. A value of $p < 0.05$ was considered statistical significance in all
208 cases. Data were expressed as mean \pm standard error of the mean (SEM) unless stated otherwise.

209 RESULTS AND DISCUSSION

210 **Extraction and modification recovery yield of AXs.** Alkaline extraction was used to investigate the
211 effect of NaOH concentration on corn bran AX extraction yields. Compared to water extraction, alkaline
212 extraction was highly efficient (Table 1). The AEAXs extraction yield increased from 2.58% up to 20.8%
213 with increasing NaOH concentration from 1% to 8%. The total AX content was determined as 26.0% of corn
214 bran (dry matter basis) using the method described by Douglas ²⁹. Thus, the AX recovery rate reached about
215 80% of the total AX using 8% NaOH. The increased AX yield achieved using alkaline treatment was likely
216 due to the ability of alkali (OH^-) to disrupt the hydrogen bonds between AXs and other components compared
217 to water treatment. Alkali may also disrupt some covalent bonds, such as ester linkages, thus loosening up
218 the cell wall matrix and solubilizing components including AXs from the cell wall of corn bran ³⁸. Following
219 extraction with 8% NaOH, the AEAX sample was modified with six types of endoxylanase treatments;
220 including P-BG, E-XYLNP and O-VR from *Thermomyces lanuginosus*, *Neocallimastix patriciarum* and
221 *Penicillium funiculosum* with 24h and 48h treatment time respectively. High recovery yields ($\approx 88\%$) of AXs
222 from the AEAX sample (dry matter basis) was achieved from enzyme treatments (Table 1).

Effects of enzyme treatments on monosaccharide composition of AXs. AEAX and E-AEAX samples

were mainly composed of arabinose (38-41%), xylose (45-47%) and galactose (14-15%). The various endoxylanase treatments had no significant effect ($p>0.05$) on the proportion of monosaccharide and A/X ratio when comparing the monosaccharide composition of AEAX with E-AEAXs. The AEAX sample extracted from corn bran using 8% NaOH had an A/X ratio of 0.82 (Table 2), which is in concordance with the results from a previous study¹⁹ that also reported the A/X ratio for alkaline extracted AXs of corn bran to be 0.82. During the modification process, GH11 endoxylanase treatments did not show obvious effects on the degree of substitution of AXs (0.82-0.89). In addition, the monosaccharide composition of E-AEAX and AEAX showed a similar proportion of galactose content and no glucose component.

Effects of enzyme treatments on molecular weight (Mw) distributions of AXs. After 24h treatment

with the three enzyme preparations, there was no difference between the Mw distribution of E-AEAXs compared with AEAX (Figure 1a). The Mw curve of E-AEAXs and AEAX following 24 hours of enzyme treatment contain a similar peak at large Mw (501KDa; $\log_{10}Mw\approx 5.7$; $avDP\approx 3800$). However, AEAXs subjected to longer enzymatic treatments (48h) demonstrated a change in Mw distribution (Figure 1b). The Mw distribution curve of the E-AEAXs following 48h of enzyme treatment contained two peaks, one in the large Mw range as found with 24h enzyme treatments and an additional peak in the small Mw range (3.98KDa; $\log_{10}Mw\approx 3.6$; $avDP\approx 30$). This longer exposure to enzymes reduced the proportion of large Mw AX in range 1 (100KDa to 794KDa) by approximately 20-32% and increased the proportion of small Mw AX in ranges

241 3 and 4 (0.1KDa to 10KDa) by 17-30% (Table 2). When comparing the three different enzymes, the E-AEAX
242 sample following O-VR treatment for 48 h (O-VR 48h) showed the largest increase in the proportion of AX
243 with small Mw (ranges 3 and 4) compared to AEAX (Table 2). These findings suggest that the duration of
244 enzyme treatment is a significant factor in the Mw modification of AEAX by endoxylanases. AXs have a
245 tendency to form macrostructures in aqueous solutions through chain aggregation and physical entanglements
246 ³⁹. The molecular size of AX is a key factor contributing to its behavior in solution. High Mw AXs can form
247 aggregations more easily and exhibit weakly elastic properties in solution compared to low molecular weight
248 fractions ⁴⁰. The Mw of the AEAX fraction (Table 2) consists largely (>80%) of high molecular weight
249 material (100KDa to 794KDa). Thus, some of AEAX may form macrostructures that inhibit the AX behavior
250 in the aqueous solution. Family GH 11 endoxylanases have a β -jelly roll structure and are considered able to
251 pass through the pores of the xylan network owing to their smaller molecular sizes ⁴¹. When the network of
252 AEAX becomes more tightknit via physical entanglements, this would form a barrier to the endoxylanase,
253 thus preventing hydrolysis of the xylan chain of the AXs. In this case, a longer treatment time (48h) would
254 be necessary to degrade the molecules of entangled AXs. Thus, improving solubility and reducing the
255 influence of AEAX aggregation should be considered when developing the modification process of AEAXs
256 in future studies. In addition, Biely, et al. ⁴² reported that AXs with a low degree of branching are more
257 susceptible to endoxylanase action. In the present study, the A/X ratio of AEAX was 0.82, which is a high
258 degree of branching and may explain why the Mw modification process using enzyme treatments required a

259 long treatment time to demonstrate effect. Furthermore, comparison of the abilities of the three types of
260 endoxylanases isolated from *Thermomyces lanuginosus*, *Neocallimastix patriciarum* and *Penicillium*
261 *funiculosum* in modifying the Mw of AEAX from corn bran, showed that the most effective one to be O-VR
262 from *Penicillium funiculosum*. From previous studies, it is important to note that due to the differences in
263 substrate specificities, binding modules and enzyme production technology, different xylanases may have
264 different activity in reducing the Mw of xylans^{21, 43}. In conclusion, enzyme modification processes altered
265 the Mw distribution of the AX extracts obtained following 48h treatment but did not appreciably change the
266 observed A/X ratio or individual monosaccharide composition. Thus, the Mw distribution was identified as
267 the major factor that differed between alkaline extracted AX and enzymatic modified AX.

268 **Effect of AX treatments on growth and viability of U937 cells.** Figures 2a and 2b demonstrate that
269 the viable and total cell count of U937 cells treated with AEAX or E-AEAX (at concentrations of 50, 500,
270 1000µg/ml) were not significantly different to those of the untreated negative control ($p>0.05$). The viability
271 of macrophages following AX treatments was over 90% in all cases. Polysaccharides extracted from plants
272 have received considerable attention due to their wide immune-modulatory activities and low toxicity⁴⁴. The
273 present study indicated that AX samples have no significant detrimental effects on cell growth and viability
274 of human U937 macrophages, even at a high AX concentration of 1000µg/ml. However, in direct contrast,
275 cell viability and growth were significantly reduced after treatment with high concentrations (500 and
276 1000µg/ml) of LPS compared to the untreated negative control ($p<0.01$). This finding is in agreement with

277 previous studies that have also shown that LPS inhibits macrophage proliferation and survival in a time- and
278 dose-dependent manner^{45,46}. Stimulation of inflammatory cells with LPS acts as a useful model to investigate
279 immune activation following bacterial infection. Vadiveloo, et al.⁴⁷ found that bacterial-derived LPS impairs
280 cell proliferation by inhibiting the expression of cyclin D1, an essential protein for cell proliferation in many
281 cell types, including macrophages.

282 **Effect of AXs on NO production by U937 cells.** AEAX and E-AEAX were assessed in terms of their
283 ability to induce NO production by U937 cells. The relationship between structure and immunomodulation
284 was investigated in U937 macrophages by assessing changes in NO secretion following treatment with a
285 range of AX concentrations and Mw distributions. Treatment with AEAX, E-AEAX or LPS at all
286 concentrations (5 to 1000µg/ml) tested significantly increased NO production (Table 3; Figure 3a and 3b) in
287 U937 cells compared with the untreated control ($p<0.05$). This finding is consistent with previous studies
288 that found AXs from other cereal sources were able to stimulate NO production in rat and human
289 macrophages^{48, 25}. At high concentrations (500-1000µg/ml) of LPS, the raw levels of NO produced by the
290 U937 cells significantly decreased ($p<0.05$) compared to levels generated by lower concentrations of LPS,
291 mirroring the substantial inhibitory effect of LPS on cell growth and viability (Figure 2c) at high
292 concentrations. Thus, when allowing for these changes in cell counts, LPS significantly increased NO
293 production per million viable cells in a dose-dependent manner (Figure 3b). NO production was significantly
294 ($P<0.05$) higher following E-AEAX treatment at low concentration ranges (5-50µg/ml) compared to AEAX

295 treatment at corresponding concentrations. NO production did not significantly increase in a dose-dependent
296 manner following E-AEAX treatment, suggesting the optimal dose range for E-AEAX was already reached
297 at the low AX concentration range (5-50µg/ml). In contrast, the AEAX treatment was most effective at
298 inducing NO production at higher concentration ranges (500-1000µg/ml) with the optimum predicted at some
299 value above 1000µg/ml by extrapolation of Figure 3a and 3b. The optimum dose ranges of AEAX and E-
300 AEAX were clearly different; AEAX induced significantly lower NO production compared to E-AEAX and
301 LPS at concentrations below 50µg/ml ($p<0.05$). Thus, this study suggests that E-AEAX is more effective
302 than AEAX as a stimulator of NO release at low (5-50µg/ml) AX concentrations. In contrast, AEAX needs
303 to be at high concentrations (>500µg/ml) to be more effective than E-AEAX at inducing NO production.

304 NO production by U937 cells was influenced by the Mw distribution of AXs. Depending on the
305 enzymatic modification process, AEAX and E-AEAX exhibited significantly different Mw distributions;
306 33.1% of E-AEAX had Mw ranging from 158Da to 10KDa (avDP=30) whereas AEAX only contained 2.82%
307 AX in this range. In addition, the branch degree (A/X) and monosaccharide composition of AEAX and E-
308 AEAX were similar. The results suggest the dose range and Mw distribution of AXs are key determinants
309 of immune-modulatory activity. Regardless of dose, corn bran AXs in both the low and high Mw range
310 significantly induced NO production compared to untreated macrophages. AXs in the low Mw range
311 (0.1KDa-10KDa) were the most effective at inducing NO secretion by U937 macrophages at low AX
312 concentration ranges (5-50 µg/ml) with NO production peaking at 62.9 ± 1.2 µM per million viable cells with

313 5 µg/ml of AX (P = 0.0009). In contrast, AXs in the high Mw range (100-794KDa) were most effective at
314 inducing NO at high AX concentration ranges (500-1000 µg/ml) with NO production reaching a maximum
315 of 62.7±1.3 µM per million viable cells at 1000 µg/ml of AX (P = 0.0011). The molecular structure of AXs
316 have already been reported to have an effect on some of their physicochemical properties in solution,
317 including tertiary conformation of the AX chain in solution, viscosity of the solution and elastic properties
318 of the solution ^{9, 49}. Therefore, future work should investigate the effect of molecular weight combined with
319 relative physicochemical properties on the immune-modulating activities of AXs.

320 Rice bran AX has been shown to induce macrophage-derived nitric oxide (NO) production in vitro in a
321 dose-dependent manner using both a murine macrophage cell line RAW264.7 and murine peritoneal
322 macrophages ²⁵. Cereal-derived AXs have also been shown to induce phagocytosis in a dose-dependent
323 manner both in the human U937 macrophage cell line and in murine peritoneal macrophages ^{25, 50}. The in
324 vitro production of pro-inflammatory cytokines tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-
325 6) are stimulated following treatment with rice bran AX in U937 human macrophages, RAW264.7 murine
326 macrophages and murine peritoneal macrophages ²⁵. This investigation in a U937 cell line supports these
327 published findings by showing AXs from corn bran are capable of directly stimulating NO production in
328 human macrophages at all concentrations investigated (5-1000 µg/ml). Macrophage-derived NO can form a
329 number of oxidation products such as NO₂, NO₂⁻ and N₂O₃ because of its highly reactive free radical structure
330 ⁵¹, and these products come into play during macrophage-mediated immune defense against numerous

331 pathogens following infection⁵². Thus, this investigation indicates a potential nutritional/prophylactic benefit
332 of dietary AXs to stimulate low (background) levels of innate immunity in the absence of infection or disease.
333 An ongoing low level of immune activity generated through dietary intake of AXs may reduce the likelihood
334 or severity of infection following subsequent exposure to pathogens. This is supported by the fact that oral
335 administration of wheat AXs (at 2500 mg/kg, n = 10) induces anti-inflammatory effects in mice treated with
336 bacterial LPS and reduces NO production in LPS-stimulated murine macrophages⁵⁰. NO production by
337 macrophages is also reported to mediate T cell responses, suppress anti-inflammatory effects and regulate of
338 leukocyte recruitment⁵³. Thus, dietary AXs may induce a wide range of immune system responses via NO
339 pathways, thereby heightening natural immunity and maintaining human health at multiple levels.

340 Further investigations are now required to determine the precise signaling mechanism through which
341 AXs induce NO production. In addition, in vivo studies are essential to determine whether the effects of dose
342 range and Mw distribution on AX-induced NO production demonstrated in this study are mirrored in the
343 body.

344

345 **ABBREVIATIONS USED**

346 AXs, arabinoxylans; AEAXs, alkaline extracted AXs; A/X, ratio of arabinose to xylose; DP, degree of
347 polymerization; E-AEAXs, enzyme-modified AEAX; E-XYLNP, Endo-1,4- β -xylanase of megazyme; GH,
348 glycoside hydrolase; LPS, lipopolysaccharide; Mw, molecular weight; NO, nitric oxide; O-VR,

349 OPTIMASH™ VR enzyme; P-BG, pentopan mono BG enzyme.

350

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REFERENCES

- 1.National Institutes of Health, N. Understanding the immune system and how it works. 2003.
- 2.Gleeson, M. Assessing immune function changes in exercise and diet intervention studies. *Curr. Opin. Clin. Nutr. Metab. Care* **2005**, *8* (5), 511-5.
- 3.Plat, J.; Mensink, R. P. Food components and immune function. *Curr. Opin. Lipidol.* **2005**, *16* (1), 31-7.
- 4.Webster Marketon, J. I.; Glaser, R. Stress hormones and immune function. *Cell. Immunol.* **2008**, *252* (1-2), 16-26.
- 5.Kaminogawa, S.; Nanno, M. Modulation of Immune Functions by Foods. *Evidence-based complementary and alternative medicine : eCAM* **2004**, *1* (3), 241-250.
- 6.Meoni, P.; Restani, P.; Mancama, D. T. Review of existing experimental approaches for the clinical evaluation of the benefits of plant food supplements on cardiovascular function. *Food Funct.* **2013**, *4* (6), 856-70.
- 7.Tzianabos, O. Polysaccharide Immunomodulators as Therapeutic Agents: Structural Aspects and Biologic Function. *Clin. Microbiol. Rev.* **2002**, *13* (4), 523-533.
- 8.Volman, J. J.; Ramakers, J. D.; Plat, J. Dietary modulation of immune function by beta-glucans. *Physiol. Behav.* **2008**, *94* (2), 276-84.
- 9.Zhang, S.; Li, W.; Smith, C. J.; Musa, H. Cereal-derived arabinoxylans as biological response modifiers: extraction, molecular features, and immune-stimulating properties. *Crit. Rev. Food Sci. Nutr.* **2015**, *55* (8), 1033-50.
- 10.Zhou, S.; Liu, X.; Guo, Y.; Wang, Q.; Peng, D.; Cao, L. Comparison of the immunological activities of arabinoxylans from wheat bran with alkali and xylanase-aided extraction. *Carbohydr. Polym.* **2010**, *81*, 784-789.
11. Kim, H. Y.; Han, J. T.; Hong, S. G.; Yang, S. B.; Hwang, S. J.; Shin, K. S.; Suh, H. J.; Park, M. H. Enhancement of immunological activity in exo-biopolymer from submerged culture of *Lentinus edodes* with rice bran. *Nat. Prod. Sci.* **2005**, *11*, 183-187.
- 12.Mendis, M.; Leclerc, E.; Simsek, S. Arabinoxylans, gut microbiota and immunity. *Carbohydr. Polym.* **2016**, *139*, 159-66.
- 13.Samuelsen, A. B.; Rieder, A.; Grimmer, S.; Michaelsen, T. E.; Knutsen, S. H. Immunomodulatory activity of dietary fiber: arabinoxylan and mixed-linked beta-

388 glucan isolated from barley show modest activities in vitro. *Int. J. Mol. Sci.* **2011**,
389 12 (1), 570-87.

390 14.Hooper, L. V.; Macpherson, A. J. Immune adaptations that maintain homeostasis
391 with the intestinal microbiota. *Nat. Rev. Immunol.* **2010**, 10 (3), 159-69.

392 15. Choi, J. Y.; Paik, D. J.; Kwon, D.Y.; Park, Y. Dietary supplementation with rice bran
393 fermented with *Lentinus edodes* increases interferon- γ activity without causing
394 adverse effects: a randomized, double-blind, placebo-controlled, parallel-group
395 study. *Nutr. J.* **2014**, 13, 35.

396 16.Zhang, Z.; Smith, C.; Li, W. Extraction and modification technology of
397 arabinoxylans from cereal by-products: A critical review. *Food Res. Int.* **2014**, 65,
398 423-436.

399 17.Yadav, M. P.; Johnston, D. B.; Hotchkiss, A. T. J.; Hicks, K. B. Corn fiber gum: A
400 potential gum arabic replacer for beverage flavor emulsification. *Food*
401 *Hydrocolloids* **2007**, 21, 1022-1030.

402 18.Doner, L. W.; Hicks, K. B. Isolation of hemicellulose from corn fiber by alkaline
403 hydrogen peroxide extraction. *Cereal Chem.* **1997**, 74, 176–181.19.Doner, L. W.;
404 Chau, H. K.; Fishman, M. L.; Hicks, K. B. An improved process for isolation of corn
405 fiber gum. *Cereal Chem.* **1998**, 75 (4), 408-411.

406 20.Paes, G.; Berrin, J. G.; Beaugrand, J. GH11 xylanases:
407 Structure/function/properties relationships and applications. *Biotechnol. Adv.*
408 **2012**, 30 (3), 564-92.

409 21.Driss, D.; Berrin, J. G.; Juge, N.; Bhiri, F.; Ghorbel, R.; Chaabouni, S. E. Functional
410 characterization of *Penicillium occitanis* Pol6 and *Penicillium funiculosum* GH11
411 xylanases. *Protein expression and purif.* **2013**, 90 (2), 195-201.

412 22.Beaugrand, J.; Chambat, G.; Wong, V. W.; Goubet, F.; Remond, C.; Paes, G.;
413 Benamrouche, S.; Debeire, P.; O'Donohue, M.; Chabbert, B. Impact and efficiency of
414 GH10 and GH11 thermostable endoxylanases on wheat bran and alkali-extractable
415 arabinoxylans. *Carbohydr. Res.* **2004**, 339 (15), 2529-40.

416 23.Elshafei, A. M.; Vega, J. L.; Klasson, K. T.; Clausen, E. C.; Gaddy, J. L. The
417 saccharification of corn stover by cellulase from *Penicillium funiculosum* *Bioresour.*
418 *Technol.* **1991**, 35 (1), 73-80.

419 24.Korhonen, R.; Lahti, A.; Kankaanranta, H.; Moilanen, E. Nitric oxide production
420 and signaling in inflammation. *Current drug targets. Inflammation and allergy*

421 **2005**, 4 (4), 471-9.

422 25.Ghoneum, M.; Matsuura, M. Augmentation of macrophage phagocytosis by
 423 modified arabinoxylan rice bran (MGN-3/biobran). *Int. J. Immunopathol.*
 424 *Pharmacol.* **2004**, 17 (3), 283-92.

425 26.Sundstrom, C.; Nillon, K. Establishment and characterization of a human
 426 histiocytic lymphoma cell line (U-937). *Int. J. Cancer* **1976**, 17, 565-577.

427 27.Li, W.; Zhang, S.; Smith, C. The molecular structure features-immune
 428 stimulatory activity of arabinoxylans derived from the pentosan faction of wheat
 429 flour. *J. Cereal Sci.* **2015**.

430 28.Malunga, L. N.; Beta, T. Antioxidant capacity of arabinoxylan oligosaccharide
 431 fractions prepared from wheat aleurone using *Trichoderma viride* or
 432 *Neocallimastix patriciarum* xylanase. *Food chem.* **2015**, 167, 311-9.

433 29.Douglas, S. G. A rapid method for the determination of pentosans in wheat flour.
 434 *Food chem.* **1980**, 7, 139-145.

435 30.Swennen, K.; Courtin, C. M.; Lindemans, G. C. J. E.; Delcour, J. A. Large-scale
 436 production and characterisation of wheat bran arabinoxyloligosaccharides. *J. Sci.*
 437 *Food Agric.* **2006**, 86, 1722-1731.

438 31.Li, W.; Hu, H.; Wang, Q.; Brennan, C. S. Molecular Features of Wheat Endosperm
 439 Arabinoxylan Inclusion in Functional Bread. *Foods* **2013**, 2 (2), 225-237.

440 32.Stoklosa, R. J.; Hodge, D. B. Extraction, recovery, and characterization of
 441 hardwood and grass hemicelluloses for integration into biorefining processes. *Ind.*
 442 *Eng. Chem. Res.* **2012**, 51 (34), 11045-11053.

443 33.Courtin, C. M.; Swennen, K.; Broekaert, W. F.; Swennen, Q.; Buyse, J. Effects of
 444 dietary inclusion of xylooligo-saccharides, arabinoxyloligosaccharides and
 445 soluble arabinoxylan on the microbial composition of caecal contents of chickens.
 446 *J. Sci. Food Agric.* **2008**, 88, 2517-2522.

447 34.Palama, I. E.; Musaro, M.; Coluccia, A. M.; D'Amone, S.; Gigli, G. Cell Uptake and
 448 Validation of Novel PECs for Biomedical Applications. *J. Drug Delivery* **2011**, 2011,
 449 203676.

450 35.Dawson, T. M.; Dawson, V. L. Nitric oxide: Actions and pathological roles.
 451 *Neuroscientist* **1995**, 7-18.

452 36.Griess, P. Bemerkungen zu der Abhandlung der H.H. Weselsky und Benedikt
 453 "Ueber einige Azoverbindungen". *Chem. Ber.* **1879**, 12, 426-428.

454 37.Jeong, H. G.; Kim, J. Y. Induction of inducible nitric oxide synthase expression by
 455 18beta-glycyrrhetic acid in macrophages. *FEBS lett.* **2002**, *513* (2-3), 208-12.

456 38.Fincher, G. B.; Stone, B. A. Cell walls and their components in cereal grain
 457 technology. In *Advances in Cereal Science and Technology*, Pomeranz, Y., Ed.
 458 American Association of Cereal Chemistry: St. Paul, 1986; pp 207-295.

459 39.Warrand, J.; Michaud, P.; Picton, L.; Muller, G.; Courtois, B.; Ralainirina, R.;
 460 Courtois, J. Contributions of intermolecular interactions between constitutive
 461 arabinoxylans to the flaxseeds mucilage properties. *Biomacromolecules* **2005**, *6*
 462 (4), 1871-6.

463 40.Izydorczyk, M. S.; Biliaderis, C. G. Effect of molecular size on physical properties
 464 of wheat arabinoxylans. *J. Agric. Food Chem.* **1992**, *40*, 561-568.

465 41.Juturu, V.; Wu, J. C. Microbial xylanases: engineering, production and industrial
 466 applications. *Biotechnol. Adv.* **2012**, *30* (6), 1219-27.

467 42.Biely, P.; Vrsanska, M.; Tenkanen, M.; Kluepfel, D. Endo-beta-1,4-xylanase
 468 families: differences in catalytic properties. *J. Biotechnol.* **1997**, *57* (1-3), 151-66.

469 43.Shoseyov, O.; Shani, Z.; Levy, I. Carbohydrate binding modules: biochemical
 470 properties and novel applications. *Microbiol. Mol. Biol. Rev.* **2006**, *70* (2), 283-95.

471 44.Schepetkin, I. A.; Quinn, M. T. Botanical polysaccharides: macrophage
 472 immunomodulation and therapeutic potential. *Int. Immunopharmacol.* **2006**, *6* (3),
 473 317-33.

474 45.Vairo, G.; Royston, A. K.; Hamilton, J. A. Biochemical events accompanying
 475 macrophage activation and the inhibition of colony-stimulating factor-1-induced
 476 macrophage proliferation by tumor necrosis factor-alpha, interferon-gamma, and
 477 lipopolysaccharide. *J. Cell. Physiol.* **1992**, *151* (3), 630-41.

478 46.Vadiveloo, P. K.; Vairo, G.; Novak, U.; Royston, A. K.; Whitty, G.; Filonzi, E. L.;
 479 Cragoe, E. J., Jr.; Hamilton, J. A. Differential regulation of cell cycle machinery by
 480 various antiproliferative agents is linked to macrophage arrest at distinct G1
 481 checkpoints. *Oncogene* **1996**, *13* (3), 599-608.

482 47.Vadiveloo, P. K.; Keramidaris, E.; Morrison, W. A.; Stewart, A. G.
 483 Lipopolysaccharide-induced cell cycle arrest in macrophages occurs
 484 independently of nitric oxide synthase II induction. *Biochim. Biophys. Acta* **2001**,
 485 *1539* (1-2), 140-6.

486 48.Nagata, J.; Higashiuesato, Y.; Maeda, G.; Chinen, I.; Saito, M.; Iwabuchi, K.; Onoe,

487 K. Effects of water-soluble hemicellulose from soybean hull on serum antibody
 488 levels and activation of macrophages in rats. *J. Agric. Food Chem.* **2001**, 49 (10),
 489 4965-70.

490 49. Izydorczyk, M. S.; Biliaderis, C. G. Arabinoxylans: Technologically and
 491 nutritionally functional plant polysaccharides. In *Functional Food Carbohydrates*,
 492 Biliaderis, C. G.; Izydorczyk, M. S., Eds. CRC Press: Boca Raton, 2007; pp 249–290.

493 50. Kang, H.; Lee, M. G.; Lee, J. K.; Choi, Y. H.; Choi, Y. S. Enzymatically-Processed
 494 Wheat Bran Enhances Macrophage Activity and Has In Vivo Anti-Inflammatory
 495 Effects in Mice. *Nutrients*. **2016**, 8(4).

496 51. Cai, H.; Huang, X.; Nie, S.; Xie, M.; Phillips, G. O.; W., C. S. Study on *Dendrobium*
 497 *officinale* O-acetyl-glucomannan (Dendronan®): Part III–Immunomodulatory
 498 activity in vitro. *Bioact. Carbohydr. Diet. Fibre* **2015**, 5 (2), 99–105.

499 52. Ekman, P.; Saarinen, M.; He, Q.; Virtala, M.; Salmi, M.; Granfors, K. Human
 500 monocytic U937 cells kill *Salmonella* in vitro by NO-independent mechanisms.
 501 *Infect. Immun.* **1999**, 67 (7), 3670-3.

502 53. Bogdan, C. The function of nitric oxide in the immune system. In *Handbook of*
 503 *Experimental Pharmacology*, Mayer, B., Ed. Springer: Heidelberg, 2000; Vol. Nitric
 504 Oxide pp 443–492.

Figure Captions/Legends

Figure 1. The molecular weight distributions of E-AEAXs generated using three different enzyme (E-XYLNP, P-BG, O-VR) treatments for 24h and 48h treatment.

Figure 2. Effects of AXs and LPS on the growth & viability of U937 cells. *a-c*: U937 cells were treated with the LPS, AEAX and E-AEAX for 24hrs; The count of viable (live) and total (viable and non-viable) cells was confirmed using the trypan blue exclusion method. The solid line ‘—’ and the dotted line ‘---’ indicate the viable and total cell count of the control (that lacks treatment with AX or LPS) respectively. The viable/total cell counts after treatment with AX or LPS were compared with the corresponding viable/total cell counts of the control using the Student’s t-test (*: p-value < 0.01). Cell counts are presented as the mean \pm SEM of six sample replicates.

Figure 3. Effect of AEAX, E-AEAX and LPS treatments on NO production by U937 cells. Panel **a** shows the raw NO production measured following treatment with AX or LPS at increasing concentrations of 5-1000 μ g/ml. Panel **b** indicates the corresponding NO production per million viable cells. The symbol * indicates that the NO production following treatment with AX or LPS was significantly ($P < 0.05$) greater to that of the untreated control. The symbol # indicates NO production significantly changed ($p < 0.05$) from previous sample dosage. The symbol @ indicates NO production with E-AEAX treatment was significantly ($p < 0.05$) different to the corresponding AEAX treatment at the same AX concentration. The symbol \$ indicates NO production with AEAX or E-AEAX treatment was significantly ($p < 0.05$) different to the corresponding treatment with LPS at the same concentration.

TABLES

Table 1. AXs extract yields from corn bran and the recovery yield of modified AX using three types of endoxylanase

Alkaline extraction	NaOH concentrations				Control	Total AX
	1%	2%	4%	8%	Water	content ^a
yield (%)	2.58±0.07	3.85±0.05	9.71±0.05	20.8±0.1	0.71±0.04	26.0±0.3
Enzyme modification	Endoxylanases					
	Treatment time	P-BG	E-XYLNP	O-VR		
recovery yield	24h	88.1±0.4	88.0±0.1	88.0±0.3		
(%)	48h	88.1±0.3	88.1±0.1	88.0±0.1		

a: Total AX content indicates original AX content in corn bran (dry base). The yields are presented as mean ± SD. All experiments were conducted in triplicate.

Table 2. The monosaccharide compositions and Mw distribution of AEAXs and E-AEAXs

Samples	Monosaccharide compositions ^a					Mw distributions ^b			
	Ara (%)	Xyl (%)	Glc(%)	Gal (%)	A/X	Range 1: 1×10 ⁵ -10 ^{5.9} (Da)	Range 2: 1×10 ⁴ -10 ⁵ (Da)	Range 3: 1×10 ³ -10 ⁴ (Da)	Range 4: 1×10 ² -10 ³ (Da)
AEAX ^c	38.3±0.5	47.0±0.6	/	14.7±0.4	0.82	83.5%	13.7%	2.82%	/
24 hour enzyme treatment (E-AEAXs 24h) ^d									
E-XYLNP	39.1±1.7	47.1±1.0	/	14.5±1.0	0.83	78.1%	19.2%	2.73%	/
P-BG	40.0±1.0	45.6±0.9	/	14.5±0.3	0.88	79.7%	17.5%	2.80%	/
O-VR	39.2±2.1	47.1±1.6	/	14.0±0.5	0.83	79.7%	17.3%	2.95%	/
48 hour enzyme treatment (E-AEAXs 48h) ^d									
E-XYLNP	41.2±1.3	45.3±0.7	/	14.5±1.0	0.89	63.7%	16.3%	17.8%	2.19%
P-BG	39.9±0.8	45.2±0.8	/	14.9±0.1	0.88	54.8%	14.4%	23.4%	7.36%
O-VR	38.8±2.3	47.3±1.6	/	13.9±0.9	0.82	51.2%	15.7%	24.5%	8.64%

Monosaccharide compositions and molecular weight (Mw) distribution of AEAXs and E-AEAXs (all experiments conducted in triplicate). *a*: The proportion of each monosaccharide in AX samples presented as mean ± standard deviation (SD); *b*: The overall Mw distribution of AXs ranged from 0.1KDa to 794KDa and was divided into four molecular weight ranges. Percentage of AXs in different Mw ranges were analyzed using the LC Data Analysis (SHIMADZU Corporation). *c*: The AEAX sample indicates AXs extracted using 8% NaOH treatment; *d*: The enzyme treatments indicate modified AEAXs using one of three different enzymes (E-XYLNP, P-BG, O-VR) for 24 hours or 48 hours.

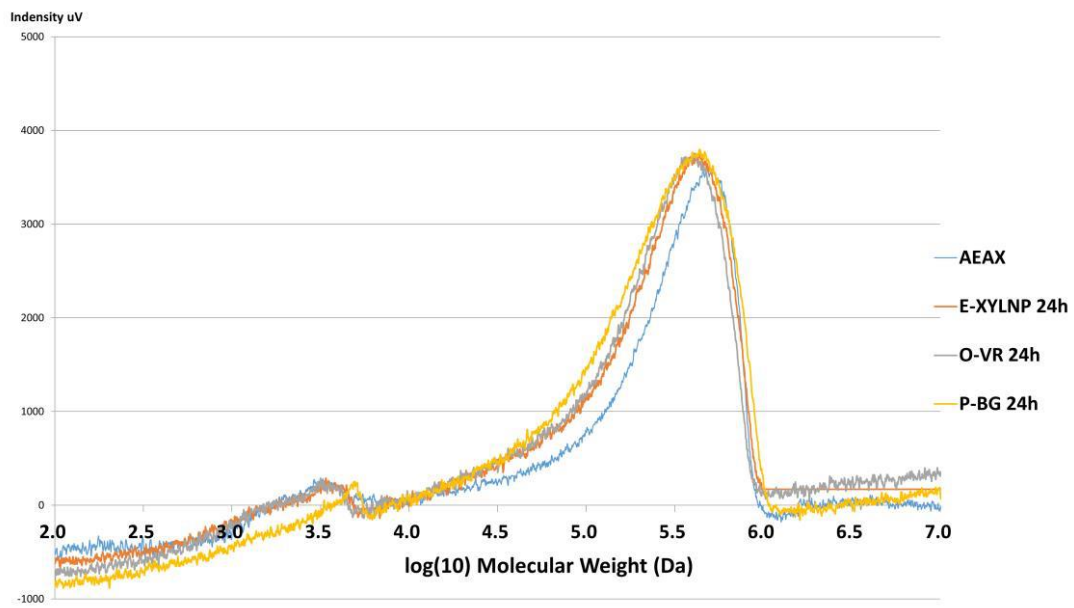
Table 3. NO production by U937 cells after 24h treatment with AEAX, E-AEAX and LPS

Sample	(a) Raw NO Production (μM)				
	(b) NO Production (μM) Per Million Viable Cells				
	Concentration of AX or LPS (μg/ml)				
	0	5	50	500	1000
LPS					
(a)	-	70.4±1.3 *	71.8±2.3 *	64.6±1.1 * #	56.5±0.1 * #
(b)	-	65.6±0.9 *	65.7±1.6 *	70.8±0.9 * #	72.2±0.0 * #
E-AEAX					
(a)	-	67.3±1.7 * @	67.7±2.4 * @	64.8±2.5 *	63.3±1.3 * \$
(b)	-	62.9±1.2 * @	62.2±1.7* @	58.6±1.7* \$	59.0±0.9* \$
	-				
AEAX					
(a)	-	57.6±1.5 * \$	61.0±2.3 * \$	65.5±2.0 * #	66.9±1.9 * # \$
(b)	-	53.7±1.1 * \$	55.9±1.6 * \$	60.2±1.4 * # \$	62.7±1.3 * # \$
Untreated					
Control					
(a)	46.1±2.0				
(b)	43.7±1.9				

The NO₂⁻ concentration (μM) (mean ± SEM) is an indication of NO production in U937 cells; The symbol * indicates NO production significantly increased (p<0.05) compared to the untreated control; The symbol # indicates NO production significantly changed (p<0.05) from previous sample dosage; The symbol @ indicates NO production with E-AEAX treatment was significantly different to the corresponding AEAX treatment at the same concentration (p<0.05); The symbol \$ indicates NO production with AEAX or E-AEAX treatment was significantly different to the corresponding treatment with LPS at the same concentration (p<0.05). The p-values indicated are those provided by a post-hoc Tukey test following an overall significant (p<0.05) difference measured by one-way ANOVA using SPSS.19. In all cases, experiments were conducted in triplicate.

Fig 1.

a.



b.

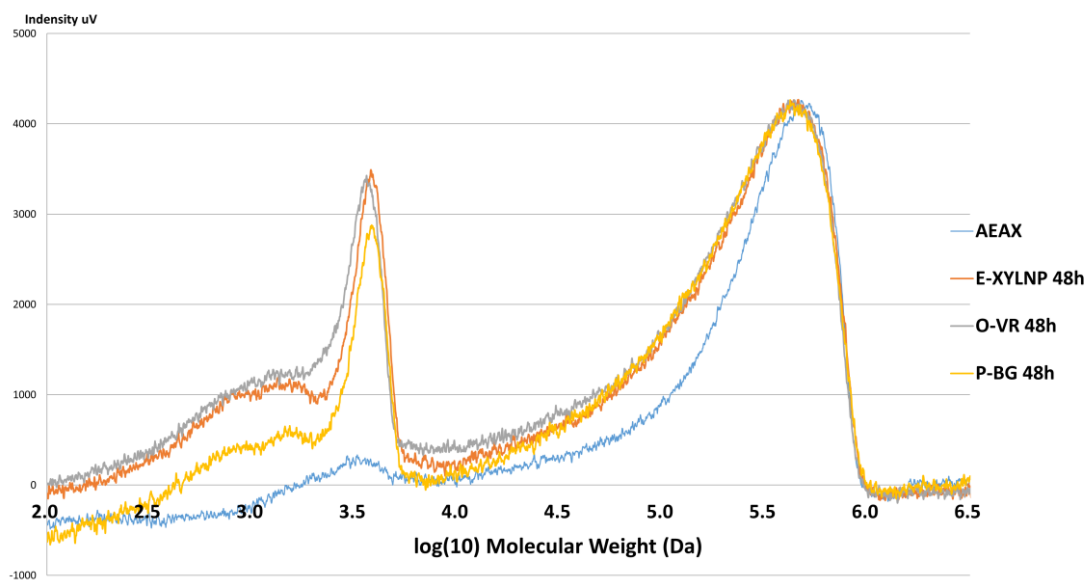
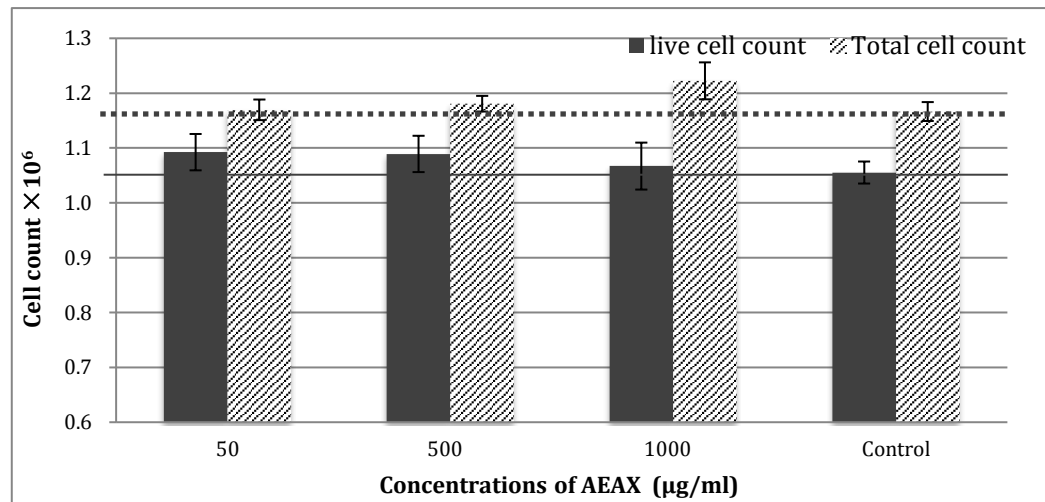
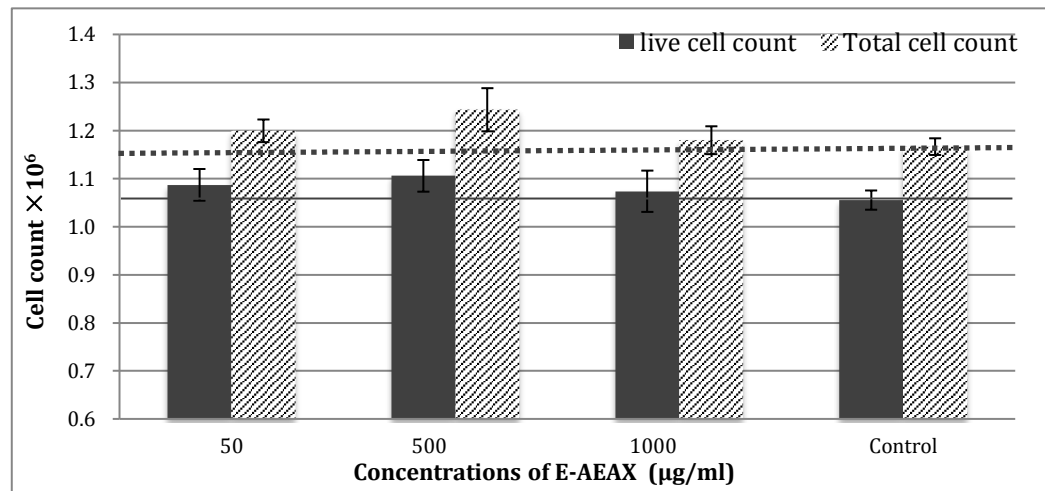


Fig.2

a.



b.



c.

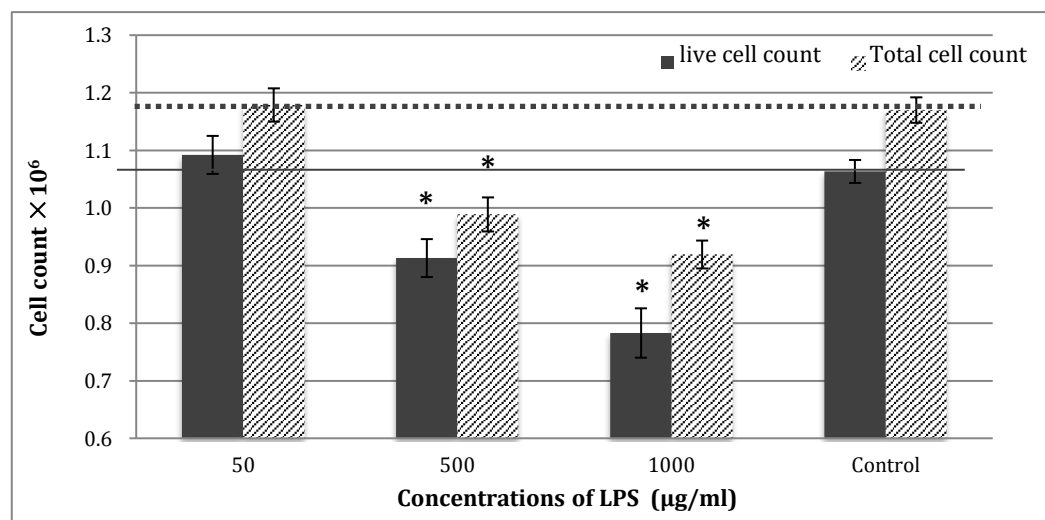
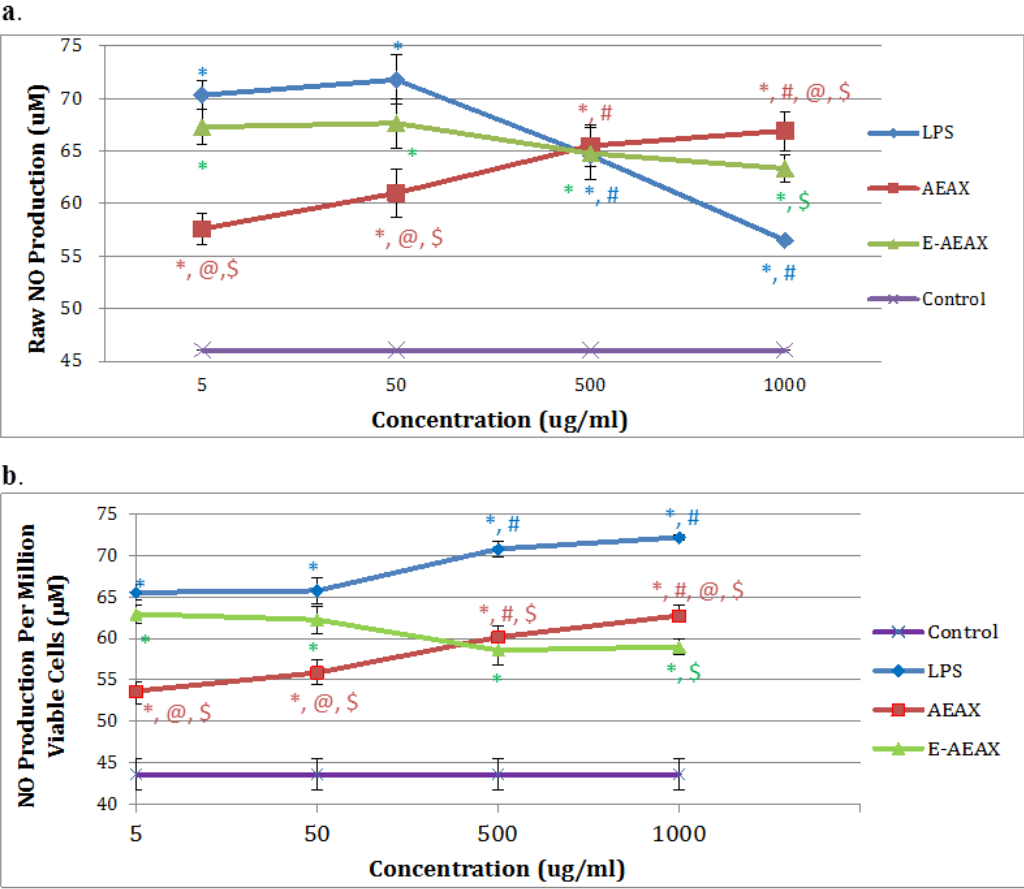


Fig 3.



*

TOC Graphic

